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| * * * | * * | * * | * * | * Welcome to STN International * * * * * * * * * | | | | | | |
|--------|-----|-------|--|---|--|--|--|--|--|--|
| NEWS | 1 | | | Web Page for STN Seminar Schedule - N. America | | | | | | |
| NEWS | 2 | MAR | 21 | IFICDB, IFIPAT, and IFIUDB enhanced with new custom | | | | | | |
| MEMO | ~ | PLAN | 31 | IPC display formats | | | | | | |
| NEWS | 3 | MAR | 31 | CAS REGISTRY enhanced with additional experimental | | | | | | |
| 112110 | 9 | | - | spectra | | | | | | |
| NEWS | 4 | MAR | 31 | CA/CAplus and CASREACT patent number format for U.S. | | | | | | |
| | | | | applications updated | | | | | | |
| NEWS | 5 | MAR | 31 | LPCI now available as a replacement to LDPCI | | | | | | |
| NEWS | 6 | MAR | 31 | EMBASE, EMBAL, and LEMBASE reloaded with enhancements | | | | | | |
| NEWS | 7 | APR | | STN AnaVist, Version 1, to be discontinued | | | | | | |
| NEWS | 8 | APR | 15 | WPIDS, WPINDEX, and WPIX enhanced with new | | | | | | |
| | | | | predefined hit display formats | | | | | | |
| NEWS | | APR | | EMBASE Controlled Term thesaurus enhanced | | | | | | |
| NEWS | | APR | | IMSRESEARCH reloaded with enhancements | | | | | | |
| NEWS | 11 | MAY | 30 | INPAFAMDB now available on STN for patent family | | | | | | |
| NEWS | 10 | MAY | 20 | searching DGENE, PCTGEN, and USGENE enhanced with new homology | | | | | | |
| NEWS | 12 | PIMI | 30 | sequence search option | | | | | | |
| NEWS | 13 | JUN | 06 | EPFULL enhanced with 260,000 English abstracts | | | | | | |
| NEWS | | JUN | | KOREAPAT updated with 41,000 documents | | | | | | |
| NEWS | | JUN | | USPATFULL and USPAT2 updated with 11-character | | | | | | |
| | | 0 011 | | patent numbers for U.S. applications | | | | | | |
| NEWS | 16 | JUN | 19 | CAS REGISTRY includes selected substances from | | | | | | |
| | | | | web-based collections | | | | | | |
| NEWS | 17 | JUN | 25 | CA/CAplus and USPAT databases updated with IPC | | | | | | |
| | | | | reclassification data | | | | | | |
| NEWS | 18 | JUN | 30 | AEROSPACE enhanced with more than 1 million U.S. | | | | | | |
| | | | | patent records | | | | | | |
| NEWS | 19 | JUN | 30 | EMBASE, EMBAL, and LEMBASE updated with additional | | | | | | |
| | | | | options to display authors and affiliated | | | | | | |
| NEWS | 0.0 | 71111 | 20 | organizations | | | | | | |
| NEWS | 20 | JUN | 30 | STN on the Web enhanced with new STN AnaVist | | | | | | |
| NEWS | 21 | JUN | 20 | Assistant and BLAST plug-in STN AnaVist enhanced with database content from EPFULL | | | | | | |
| NEWS | | JUL | | CA/CAplus patent coverage enhanced | | | | | | |
| NEWS | | JUL | | EPFULL enhanced with additional legal status | | | | | | |
| 112110 | | 001 | | information from the epoline Register | | | | | | |
| NEWS | 24 | JUL | 28 | IFICDB, IFIPAT, and IFIUDB reloaded with enhancements | | | | | | |
| NEWS | | JUL | | STN Viewer performance improved | | | | | | |
| NEWS | 26 | AUG | 01 | INPADOCDB and INPAFAMDB coverage enhanced | | | | | | |
| NEWS | EXP | RESS | JUNE 27 08 CURRENT WINDOWS VERSION IS V8.3, | | | | | | | |
| | | | AND CURRENT DISCOVER FILE IS DATED 23 JUNE 2008. | | | | | | | |
| | | | | | | | | | | |
| NEWS | | | | N Operating Hours Plus Help Desk Availability | | | | | | |
| NEWS | LOG | IN | We. | lcome Banner and News Items | | | | | | |
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NEWS IPC8 For general information regarding STN implementation of IPC 8

Enter NEWS followed by the item number or name to see news on that specific topic.

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FILE 'HOME' ENTERED AT 02:01:25 ON 11 AUG 2008

=> file medline, agricola, caba, caplus, biosis, biotechno
COST IN U.S. DOLLARS SINCE FILE TOTAL
ENTRY SESSION
FULL ESTIMATED COST 0.21
0.21

FILE 'MEDLINE' ENTERED AT 02:01:35 ON 11 AUG 2008

FILE 'AGRICOLA' ENTERED AT 02:01:35 ON 11 AUG 2008

FILE 'CABA' ENTERED AT 02:01:35 ON 11 AUG 2008 COPYRIGHT (C) 2008 CAB INTERNATIONAL (CABI)

FILE 'CAPLUS' ENTERED AT 02:01:35 ON 11 AUG 2008
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FILE 'BIOTECHNO' ENTERED AT 02:01:35 ON 11 AUG 2008 COPYRIGHT (C) 2008 Elsevier Science B.V., Amsterdam. All rights reserved.

=> s (frankard, v? or frankard v?)/au

L1 138 (FRANKARD, V? OR FRANKARD V?)/AU

11 (CYCLIN(W) DEPENDENT(W) KINASE(W) D) OR CDKD OR (D(W) 17PE(W) CYCLIN(W) DEPENDENT(W) KINASE(W) 3)

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PROCESSING COMPLETED FOR L3
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=> d 14 1-2 bib

L4 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2008 ACS on STN

AN 2007:951653 CAPLUS

DN 147:465214

TI Novel Plant-specific Cyclin-dependent Kinase Inhibitors Induced by Biotic and Abiotic Stresses

AU Peres, Adrian; Churchman, Michelle L.; Hariharan, Srivaidehirani; Himanen,

Kristiina; Verkest, Aurine; Vandepoele, Klaas; Magyar, Zoltan; Hatzfeld, Yves; Van Der Schueren, Els; Beemster, Gerrit T. S.; Frankard, Valerie; Larkin, John C.; Inze, Dirk; De Veylder, Lieven

CS CropDesign N.V., Ghent, B-9052, Belg.

SO Journal of Biological Chemistry (2007), 282(35), 25588-25596 CODEN: JBCHA3; ISSN: 0021-9258

PB American Society for Biochemistry and Molecular Biology

DT Journal

LA English

RE.CNT 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L4 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2008 ACS on STN

AN 2005:984096 CAPLUS

DN 143:280477

TI Protein and cDNA sequences of a Arabidopsis thaliana protein (cyclin-dependent) kinase CDKD and use for increasing plant seed yield

IN Frankard, Valerie

PA Cropdesign N. V., Belg.

SO PCT Int. Appl., 39 pp.

CODEN: PIXXD2

DT Patent LA English

LA Englist FAN.CNT 1

| | | | | | | KIND DATE | | | APPLICATION NO. | | | | | | | | | | |
|------|--|---|--|--|--|--|--|---|---|---|--|--|--|--|--|--|--|--|----|
| PI | WO | 2005 | 0830 | 94 | | | | 2005 | 0050909 WO 2005-EP50874 | | | | | | | | | | |
| | ,,, | W: | AE, CN, GE, LK, NO, SY, BW, AZ, EE, RO, | AG, CO, GH, LR, NZ, TJ, GH, BY, ES, SE, | AL, CR, GM, LS, OM, TM, GM, KG, FI, SI, | AM, CU, HR, LT, PG, TN, KE, KZ, FR, SK, | AT, CZ, HU, LU, PH, TR, LS, MD, GB, TR, | AU, DE, ID, LV, PL, TT, MW, RU, GR, | AZ, DK, IL, MA, PT, TZ, MZ, TJ, HU, | BA, DM, IN, MD, RO, UA, NA, TM, IE, | DZ, IS, MG, RU, UG, SD, AT, IS, | BG, EC, JP, MK, SC, US, SL, BE, IT, CI, | EE, KE, MN, SD, UZ, SZ, BG, LT, | EG, KG, MW, SE, VC, TZ, CH, LU, | ES, KP, MX, SG, VN, UG, CY, MC, | FI, KR, MZ, SK, YU, ZM, CZ, NL, | GB, KZ, NA, SL, ZA, ZW, DE, PL, | GD, LC, NI, SM, ZM, AM, DK, PT, | ZW |
| | | | | | | A1 | 20050909 | | | AU 2005-217156 CA 2005-2557375 | | | | | | | | | |
| | EP | 1723 R: | AT, | BE, | BG, | CH, | CY, | CZ, | DE, | DK, | EE, | ES, | FI, | FR, | GB, | GR, | | | |
| PRAI | BR JP MX IN US EP US | N 1946848 R 2005008322 P 2007525229 C 2006PA09645 N 2006CN03166 | | | · | A T A A A1 A | 20070724 20070906 20070416 20070608 20070614 20040301 20040305 | | CN 2005-80012292 BR 2005-8322 JP 2007-501277 MX 2006-PA9645 IN 2006-CN3166 US 2006-591095 | | | , | 20050301 20050301 20050301 20050301 20060824 20060831 | | | | | | |

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(FILE 'HOME' ENTERED AT 02:01:25 ON 11 AUG 2008)

FILE 'MEDLINE, AGRICOLA, CABA, CAPLUS, BIOSIS, BIOTECHNO' ENTERED AT 02:01:35 ON 11 AUG 2008

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1.2
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T. 4
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=> s 12 not 11
           69 L2 NOT L1
L5
=> s 15 and (plant or plants)
            25 L5 AND (PLANT OR PLANTS)
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KEEP DUPLICATES FROM MORE THAN ONE FILE? Y/(N):n
PROCESSING COMPLETED FOR L6
              8 DUPLICATE REMOVE L6 (17 DUPLICATES REMOVED)
=> d 17 1-8 ti
     ANSWER 1 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
ΤI
     Light-dependent regulation of cell division in Ostreococcus: evidence for
     a major transcriptional input
     ANSWER 2 OF 8
                      MEDLINE on STN
                                                        DUPLICATE 1
     Diverse phosphoregulatory mechanisms controlling cyclin-dependent
     kinase-activating kinases in Arabidopsis.
     ANSWER 3 OF 8
                      MEDLINE on STN
                                                        DUPLICATE 2
TI
     Control of cell division and transcription by cyclin-dependent
     kinase-activating kinases in plants.
     ANSWER 4 OF 8 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
     Genome-wide analysis of core cell cycle genes in the unicellular green
ΤI
     alga Ostreococcus tauri.
L7
     ANSWER 5 OF 8 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
TΙ
     Functional analysis of Arabidopsis CDK-activating kinases.
     ANSWER 6 OF 8
                      MEDLINE on STN
ΤТ
     The plant-specific kinase CDKF; 1 is involved in activating
     phosphorylation of cyclin-dependent kinase-activating kinases in
     Arabidopsis.
L7
     ANSWER 7 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
TΙ
     Genome-wide analysis of core cell cycle genes in Arabidopsis
     ANSWER 8 OF 8
                      MEDLINE on STN
                                                       DUPLICATE 4
TI
     CDK-related protein kinases in plants.
=> d 17 1-8 bib
     ANSWER 1 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
     2007:827616 CAPLUS
AN
DN
     147:317906
     Light-dependent regulation of cell division in Ostreococcus: evidence for
     a major transcriptional input
```

Bouget, Francois-Yvee Unite Mixte de Recherche 7628 Centre National de la Recherche Scientifique, Laboratoire Arago, Universite Paris VI, Banyuls sur Mer,

AU

Moulager, Mickael; Monnier, Annabelle; Jesson, Beline; Bouvet, Regis;

Mosser, Jean; Schwartz, Christian; Garnier, Lionel; Corellou, Florence;

```
66650, Fr.
    Plant Physiology (2007), 144(3), 1360-1369
SO
     CODEN: PLPHAY; ISSN: 0032-0889
PB
    American Society of Plant Biologists
DT
    Journal
LA
    English
              THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE.CNT 24
              ALL CITATIONS AVAILABLE IN THE RE FORMAT
     ANSWER 2 OF 8
L7
                      MEDLINE on STN
                                                        DUPLICATE 1
AN
     2006500107
                   MEDLINE
DN
     PubMed ID: 16856985
TΙ
     Diverse phosphoregulatory mechanisms controlling cyclin-dependent
     kinase-activating kinases in Arabidopsis.
AII
     Shimotohno Akie; Ohno Ryoko; Bisova Katerina; Sakaguchi Norihiro; Huang
     Jirong; Koncz Csaba; Uchimiya Hirofumi; Umeda Masaaki
CS
     Institute of Molecular and Cellular Biosciences, The University of Tokyo,
     Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan.
SO
     The Plant journal: for cell and molecular biology, (2006 Sep) Vol. 47,
     No. 5, pp. 701-10. Electronic Publication: 2006-07-11.
     Journal code: 9207397. ISSN: 0960-7412.
CY
     England: United Kingdom
DT
     Journal; Article; (JOURNAL ARTICLE)
     (RESEARCH SUPPORT, NON-U.S. GOV'T)
     English
LA
FS
     Priority Journals
EM
     200611
ED
     Entered STN: 23 Aug 2006
     Last Updated on STN: 14 Nov 2006
     Entered Medline: 13 Nov 2006
    ANSWER 3 OF 8
                      MEDLINE on STN
                                                       DUPLICATE 2
     2005533280
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AN
    PubMed ID: 16024551
DN
TI
     Control of cell division and transcription by cyclin-dependent
     kinase-activating kinases in plants.
AU
    Umeda Masaaki; Shimotohno Akie; Yamaguchi Masatoshi
CS
     Institute of Molecular and Cellular Biosciences, The University of Tokyo,
     Yayoi 1-1-1, Bunkyo-ku, Tokyo, 113-0032 Japan.. mumeda@iam.u-tokyo.ac.jp
SO
     Plant & cell physiology, (2005 Sep) Vol. 46, No. 9, pp. 1437-42.
     Electronic Publication: 2005-07-15. Ref: 48
     Journal code: 9430925, ISSN: 0032-0781,
CY
     Japan
DT
    Journal; Article; (JOURNAL ARTICLE)
     (RESEARCH SUPPORT, NON-U.S. GOV'T)
     General Review; (REVIEW)
T.A
    English
FS
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    200602
EM
ED
     Entered STN: 7 Oct 2005
     Last Updated on STN: 7 Feb 2006
     Entered Medline: 6 Feb 2006
L7
     ANSWER 4 OF 8 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
     2005:192429 BIOSIS
AN
DN
     PREV200500193729
     Genome-wide analysis of core cell cycle genes in the unicellular green
     alga Ostreococcus tauri.
```

Robbens, Steven; Khadaroo, Basheer; Camasses, Alain; Derelle, Evelyne; Ferraz, Conchita; Inze, Dirk; Van de Peer, Yves; Moreau, Herve [Reprint

Lab Arago Modeles Biol Cellulaire and Evolut, Univ Paris 06, Banyuls sur

AΠ

Authorl

Mer, France

h.moreau@obs-banvuls.fr

Molecular Biology and Evolution, (March 2005) Vol. 22, No. 3, pp. 589-597. SO CODEN: MBEVEO. ISSN: 0737-4038.

Article

- LA English
- Entered STN: 25 May 2005
- Last Updated on STN: 25 May 2005
- L7 ANSWER 5 OF 8 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN
- AN 2005:484191 BIOSIS
- DN PREV200510288190
- ΤТ Functional analysis of Arabidopsis CDK-activating kinases.
- AU Sakaguchi, Norihiro [Reprint Author]; Shimotohno, Akie; Uchimiya, Hirofumi; Sakaguchi, Kengo; Umeda, Masaaki
- Sci Univ Tokyo, Dept Appl Biol Sci, Fac Sci and Technol, Tokyo 162, Japan
- SO Plant and Cell Physiology, (2005) Vol. 46, No. Suppl. S, pp. S224. Meeting Info.: 46th Annual Meeting of the Japanese-Society-of-Plant-Physiologists. Niigata, JAPAN. March 24 -26, 2005. Japanese Soc Plant Physiologists.

CODEN: PCPHA5. ISSN: 0032-0781.

- DT Conference: (Meeting)
 - Conference; Abstract; (Meeting Abstract)
- English LA
- Entered STN: 16 Nov 2005 ED Last Updated on STN: 16 Nov 2005
- L7 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 3
- MEDLINE AN 2004551655
- DN PubMed ID: 15486101
- TΙ The plant-specific kinase CDKF; 1 is involved in activating
- phosphorylation of cyclin-dependent kinase-activating kinases in Arabidopsis. AU Shimotohno Akie; Umeda-Hara Chikage; Bisova Katerina; Uchimiya Hirofumi;
- Umeda Masaaki CS Institute of Molecular and Cellular Biosciences, University of Tokyo,
- Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-0032, Japan. SO The Plant cell, (2004 Nov) Vol. 16, No. 11, pp. 2954-66. Electronic Publication: 2004-10-14.
 - Journal code: 9208688. ISSN: 1040-4651.
- CY United States
- DT Journal: Article: (JOURNAL ARTICLE)
- (RESEARCH SUPPORT, NON-U.S. GOV'T) LA English
- FS
- Priority Journals
- OS GENBANK-AB051072
- EM 200503
- ED Entered STN: 4 Nov 2004 Last Updated on STN: 23 Mar 2005

Entered Medline: 22 Mar 2005

- L7 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2008 ACS on STN
- 2002:351745 CAPLUS AN
- DN 137:180526
- Genome-wide analysis of core cell cycle genes in Arabidopsis
- AU Vandepoele, Klaas; Raes, Jeroen; De Veylder, Lieven; Rouze, Pierre; Rombauts, Stephane; Inze, Dirk
- CS Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology (VIB), Ghent University, Ghent, B-9000, Belg.
- Plant Cell (2002), 14(4), 903-916 SO CODEN: PLCEEW; ISSN: 1040-4651

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PB
    American Society of Plant Biologists
DT
    Journal
T.A
    English
RE.CNT 59
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                      MEDLINE on STN
AN
    2001059738
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    PubMed ID: 11089864
TI
     CDK-related protein kinases in plants.
AU
     Joubes J; Chevalier C; Dudits D; Heberle-Bors E; Inze D; Umeda M; Renaudin
     JP
CS
     Laboratory of Plant Physiology, National Institute for Agronomic Research
     INRA, Villenave d'Ornon, France.
SO
     Plant molecular biology, (2000 Aug) Vol. 43, No. 5-6, pp. 607-20. Ref: 83
     Journal code: 9106343. ISSN: 0167-4412.
CY
    Netherlands
DT
     Journal; Article; (JOURNAL ARTICLE)
     General Review; (REVIEW)
LA
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    Priority Journals
EM
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ED
    Entered STN: 22 Mar 2001
     Last Updated on STN: 22 Mar 2001
     Entered Medline: 28 Dec 2000
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L2
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L4
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L6
             25 S L5 AND (PLANT OR PLANTS)
             8 DUPLICATE REMOVE L6 (17 DUPLICATES REMOVED)
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             1 CAK3AT
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T.R
TI
     Differential phosphorylation activities of CDK-activating kinases in
     Arabidopsis thaliana
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     ANSWER 1 OF 1 CAPLUS COPYRIGHT 2008 ACS on STN
AN
     2003:32075 CAPLUS
DN
     138:299760
     Differential phosphorylation activities of CDK-activating kinases in
     Arabidopsis thaliana
AΠ
     Shimotohno, Akie; Matsubayashi, Satoko; Yamaguchi, Masatoshi; Uchimiya,
     Hirofumi; Umeda, Masaaki
    Institute of Molecular and Cellular Biosciences, The University of Tokyo,
```

Bunkyo-ku, Tokyo, 113-0032, Japan

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SO FEBS Letters (2003), 534(1-3), 69-74
    CODEN: FEBLAL; ISSN: 0014-5793
PB
   Elsevier Science B.V.
DT Journal
T.A
   English
RE.CNT 37
             THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD
             ALL CITATIONS AVAILABLE IN THE RE FORMAT
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ST
    phosphorylation CDK activating kinase Arabidopsis thaliana CAK4At
     CAK3At CAK2At; protein sequence CDK activating kinase Arabidopsis
     thaliana
     372092-80-3, Protein kinase
    RL: BSU (Biological study, unclassified); PRP (Properties); BIOL
     (Biological study)
        (CAK2At, CAK3At, CAK4At; differential phosphorylation
        activities of CDK-activating kinases in Arabidopsis thaliana)
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L4
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L5
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            25 S L5 AND (PLANT OR PLANTS)
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L7
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L8
             1 S CAK3AT
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                                                                 TOTAL
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FULL ESTIMATED COST
                                                                 60.08
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CA INDEXING COPYRIGHT (C) 2008 AMERICAN CHEMICAL SOCIETY (ACS)
FILE COVERS 1971 TO PATENT PUBLICATION DATE: 7 Aug 2008 (20080807/PD)
FILE LAST UPDATED: 7 Aug 2008 (20080807/ED)
HIGHEST GRANTED PATENT NUMBER: US7409722
HIGHEST APPLICATION PUBLICATION NUMBER: US20080189819
CA INDEXING IS CURRENT THROUGH 7 Aug 2008 (20080807/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 7 Aug 2008 (20080807/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Jun 2008
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Jun 2008
USPATFULL now includes complete International Patent Classification (IPC)
reclassification data for the second quarter of 2008.
=> s 11
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17 (FRANKARD, V? OR FRANKARD V?)/AU

T.9

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1.10
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L10 ANSWER 1 OF 1 USPATFULL on STN
       2007:156672 USPATFULL
AN
       Plants having increased yield and method for making the same
       Frankard, Valerie, Sint-Genesius-Rode, BELGIUM
IN
PA
       CropDesign N.V., Zwijnaarde, BELGIUM, B-9052 (non-U.S. corporation)
ΡI
       US 20070136894
                          A1 20070614
                           A1 20050301 (10)
ΑI
       US 2005-591095
       WO 2005-EP50874
                               20050301
                               20060920 PCT 371 date
     EP 2004-100814
                           20040301
PRAI
      US 2004-550918P
                           20040305 (60)
DT
      Utility
FS
      APPLICATION
LREP
       CONNOLLY BOVE LODGE & HUTZ, LLP, P O BOX 2207, WILMINGTON, DE, 19899, US
CLMN
     Number of Claims: 24
ECL
      Exemplary Claim: 1
     4 Drawing Page(s)
LN.CNT 1396
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
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         78359 KINASE
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             5 CDKD
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        78359 KINASE
       5205913 3
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        291578 PLANT
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L13 ANSWER 1 OF 2 USPATFULL on STN
ΤI
       Transgenic plants with enhanced agronomic traits
L13 ANSWER 2 OF 2 USPATFULL on STN
       Isolated nucleic acid molecules encoding P57KIP2
=> d 113 hib
L13 ANSWER 1 OF 2 USPATFULL on STN
AN 2008:169773 USPATFULL
       Transgenic plants with enhanced agronomic traits
       Abad, Mark Scott, Webster Grove, MO, UNITED STATES
IN
PΙ
       US 20080148432 A1 20080619
AΙ
       US 2005-374300
                          A1 20051221 (11)
       Utility
DT
FS
       APPLICATION
LREP
      MONSANTO COMPANY, 800 N. LINDBERGH BLVD., ATTENTION: GAIL P. WUELLNER,
       IP PARALEGAL, (E2NA), ST. LOUIS, MO, 63167, US
CLMN
      Number of Claims: 11
ECL
      Exemplary Claim: 1-22
DRWN
      3 Drawing Page(s)
LN.CNT 5060
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
=> d 113 kwic
L13 ANSWER 1 OF 2 USPATFULL on STN
TΙ
       Transgenic plants with enhanced agronomic traits
AB
       This invention provides transgenic plant cells with
       recombinant DNA for expression of proteins that are useful for imparting
       enhanced agronomic trait(s) to transgenic crop plants. This
       invention also provides transgenic plants and progeny seed
       comprising the transgenic plant cells where the plants
       are selected for having an enhanced trait selected from the group of
       traits consisting of enhanced water use efficiency, enhanced. . .
       enhanced nitrogen use efficiency, enhanced seed protein and enhanced
       seed oil. Also disclosed are methods for manufacturing transgenic seed
       and plants with enhanced traits.
SUMM
      Disclosed herein are inventions in the field of plant genetics
       and developmental biology. More specifically, the present inventions
       provide plant cells with recombinant DNA for providing an
       enhanced trait in a transgenic plant, plants
       comprising such cells, seed and pollen derived from such plants
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, methods of making and using such cells, plants, seeds and

pollen.

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SUMM
      Transgenic plants with improved agronomic traits such as
       yield, environmental stress tolerance, pest resistance, herbicide
       tolerance, improved seed compositions, and the like are desired by both
       farmers and consumers. Although considerable efforts in plant
       breeding have provided significant gains in desired traits, the ability
       to introduce specific DNA into plant genomes provides further
       opportunities for generation of plants with improved and/or
       unique traits. Merely introducing recombinant DNA into a plant
       genome doesn't always produce a transgenic plant with an
       enhanced agronomic trait. Methods to select individual transgenic events
       from a population are required to identify those transgenic. .
      This invention employs recombinant DNA for expression of proteins that
SUMM
      are useful for imparting enhanced agronomic traits to the transgenic
      plants. Recombinant DNA in this invention is provided in a
       construct comprising a promoter that is functional in plant
       cells and that is operably linked to DNA that encodes a protein having
       at least one amino acid domain in. . . of Pfam domain names as
       identified in Table 12. In more specific embodiments of the invention
       the protein expressed in plant cells has an amino acid
       sequence with at least 90% identity to a consensus amino acid sequence
       in the group. . . 1482 and homologs thereof listed in Table 2. In
       even more specific embodiments of the invention the protein expressed in
       plant cells is a protein selected from the group of proteins
       identified in Table 1.
SUMM
      Other aspects of the invention are specifically directed to transgenic
       plant cells comprising the recombinant DNA of the invention,
       transgenic plants comprising a plurality of such plant
       cells, progeny transgenic seed, embryo and transgenic pollen from such
       plants. Such plant cells are selected from a
       population of transgenic plants regenerated from plant
       cells transformed with recombinant DNA and that express the protein by
       screening transgenic plants in the population for an enhanced
       trait as compared to control plants that do not have said
       recombinant DNA, where the enhanced trait is selected from group of
       enhanced traits consisting of. . .
SUMM
     In yet another aspect of the invention the plant cells,
      plants, seeds, embryo and pollen further comprise DNA expressing
       a protein that provides tolerance from exposure to an herbicide applied
       at levels that are lethal to a wild type of said plant cell.
       Such tolerance is especially useful not only as a advantageous trait in
       such plants but is also useful in a selection step in the
      methods of the invention. In aspects of the invention the. .
      Yet other aspects of the invention provide transgenic plants
       which are homozygous for the recombinant DNA and transgenic seed of the
       invention from corn, soybean, cotton, canola, alfalfa, wheat or rice
       plants. In other important embodiments for practice of various
       aspects of the invention in Argentina the recombinant DNA is provided in
      plant cells derived from corn lines that that are and maintain
      resistance to the Mal de Rio Cuarto virus or the. . .
      This invention also provides methods for manufacturing non-natural,
SUMM
       transgenic seed that can be used to produce a crop of transgenic
       plants with an enhanced trait resulting from expression of
       stably-integrated, recombinant DNA for expressing a protein having at
       least one domain. . . in the group of Pfam names identified in Table
       12. More specifically the method comprises (a) screening a population of
       plants for an enhanced trait and a recombinant DNA, where
       individual plants in the population can exhibit the trait at a
       level less than, essentially the same as or greater than the level that
       the trait is exhibited in control plants which do not express
       the recombinant DNA, (b) selecting from the population one or more
       plants that exhibit the trait at a level greater than the level
       that said trait is exhibited in control plants, (c) verifying
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that the recombinant DNA is stably integrated in said selected plants, (d) analyzing tissue of a selected plant to determine the production of a protein having the function of a protein encoded by nucleotides in a sequence of one of SEQ ID NO: 1-741; and (e) collecting seed from a selected plant. In one aspect of the invention the plants in the population further comprise DNA expressing a protein that provides tolerance to exposure to an herbicide applied at levels that are lethal to wild type plant cells and the selecting is effected by treating the population with the herbicide, e.g. a glyphosate, dicamba, or glufosinate compound. In another aspect of the invention the plants are selected by identifying plants with the enhanced trait. The methods are especially useful for manufacturing corn, soybean, cotton, alfalfa, wheat or rice seed.

SUMM the invention provides a method of producing hybrid corn seed comprising acquiring hybrid corn seed from a herbicide tolerant corn plant which also has stably-integrated, recombinant DNA comprising a promoter that is (a) functional in plant cells and (b) is operably linked to DNA that encodes a protein having at least one domain of amino acids. . . by a Pfam name in the group of Pfam names identified in Table 12. The methods further comprise producing corn plants from said hybrid corn seed, wherein a fraction of the plants produced from said hybrid corn seed is homozygous for said recombinant DNA, a fraction of the plants produced from said hybrid corn seed is hemizygous for said recombinant DNA, and a fraction of the plants produced from said hybrid corn seed has none of said recombinant DNA; selecting corn plants which are homozygous and hemizygous for said recombinant DNA by treating with an herbicide; collecting seed from herbicide-treated-surviving corn plants and planting said seed to produce further progeny corn plants; repeating the selecting and collecting steps at least once to produce an inbred corn line; and crossing the inbred corn.

SUMM Another aspect of the invention provides a method of selecting a plant comprising plant cells of the invention by using an immunoreactive antibody to detect the presence of protein expressed by recombinant DNA in seed or plant tissue. Yet another aspect of the invention provides anti-counterfeit milled seed having, as an indication of origin, a plant cells of this invention.

Still other aspects of this invention relate to transgenic plants with enhanced water use efficiency or enhanced nitrogen use efficiency. For instance, this invention provides methods of growing a corn, cotton or soybean crop without irrigation water comprising planting seed having plant cells of the invention which are selected for enhanced water use efficiency. Alternatively methods comprise applying reduced irrigation water, e.g.. . invention also provides methods of growing a corn, cotton or soybean crop without added nitrogen fertilizer comprising planting seed having plant cells of the invention which are selected for enhanced nitrogen use efficiency.

DETD As used herein a "plant cell" means a plant cell that is transformed with stably-integrated, non-natural, recombinant DNA, e.g. by Agrobacterium-mediated transformation or by baombardment using microparticles coated with recombinant DNA or other means. A plant cell of this invention can be an originally-transformed plant cell that exists as a microorganism or as a progeny plant cell that is regenerated into differentiated tissue, e.g. into a transgenic plant with stably-integrated, non-natural recombinant DNA, or seed or pollen derived from a progeny transgenic plant.

DETD As used herein a "transgenic plant" means a plant whose genome has been altered by the stable integration of recombinant DNA. A transgenic plant includes a plant regenerated

from an originally-transformed plant cell and progeny transgenic plants from later generations or crosses of a transformed plant.

- DETD . . . function, e.g. proteins that belong to the same Pfam protein family and that provide a common enhanced trait in transgenic plants of this invention. Homologs are expressed by homologous genes. Homologous genes include naturally occurring alleles and artificially-created variants. Degeneracy of. . . identity over the full length of a protein identified as being associated with imparting an enhanced trait when expressed in plant cells. Homologs include proteins with an amino acid sequence that has at least 90% identity to a consensus amino acid. .
- DETD . lysine-arginine, alanine-valine, aspartic acid-glutamic acid, and asparagine-glutamine. A further aspect of the homologs encoded by DNA useful in the transgenic plants of the invention are those proteins that differ from a disclosed protein as the result of deletion or insertion of.
- DETD . . be low. Once one DNA is identified as encoding a protein which imparts an enhanced trait when expressed in transgenic plants, other DNA encoding proteins in the same protein family are identified by querying the amino acid sequence of protein encoded. . . in the protein family and have cognate DNA that is useful in constructing recombinant DNA for the use in the plant cells of this invention. Hidden Markov Model databases for use with HMMER software in identifying DNA expressing protein in a common Pfam for recombinant DNA in the plant cells of this invention are also included in the appended computer listing. The HMMER software and Pfam databases are version. . . the gathering cutoff disclosed in Table 12 by Pfam analysis disclosed herein can be used in recombinant DNA of the plant cells of this invention, e.g. for selecting transgenic plants having enhanced agronomic traits. The relevant Pfams for use in this invention, as more specifically disclosed below, are bZIP.sub.--1, bZIP.sub.--2,. .
- DETD As used herein "promoter" means regulatory DNA for initializing transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells whether or not its origin is a plant cell, e.g. is it well known that Agrobacterium promoters are functional in plant cells. Thus, plant promoters include promoter DNA obtained from plants, plant viruses and bacteria such as Agrobacterium and Bradyrhizobium bacteria. Examples of promoters under developmental control include promoters that preferentially initiate.
- DETD As used herein "expressed" means produced, e.g. a protein is expressed in a plant cell when its cognate DNA is transcribed to mRNA that is translated to the protein.
- DETD As used herein a "control plant" means a plant that does not contain the recombinant DNA that expressed a protein that impart an enhanced trait. A control plant is to identify and select a transgenic plant that has an enhance trait. A suitable control plant can be a non-transgenic plant of the parental line used to generate a transgenic plant, i.e. devoid of recombinant DNA. A suitable control plant may in some cases be a progeny of a hemizygous transgenic plant line that is does not contain the recombinant DNA, known as a negative segregant.
- DETD As used herein an "enhanced trait" means a characteristic of a transgenic plant that includes, but is not limited to, an enhance agronomic trait characterized by enhanced plant morphology, physiology, growth and development, yield, nutritional enhancement, disease or pest resistance, or environmental or chemical tolerance. In more specific. . . infestation, nematode infestation,

cold temperature exposure, heat exposure, osmotic stress, reduced nitrogen nutrient availability, reduced phosphorus nutrient availability and high plant density. "Yield" can be affected by many properties including without limitation, plant height, pod number, pod position on the plant, number of internodes, incidence of pod shatter, grain size, efficiency of nodulation and nitrogen fixation, efficiency of nutrient assimilation, resistance to biotic and abiotic stress, carbon assimilation, plant architecture, resistance to lodging, percent seed germination, seedling vigor, and juvenile traits. Yield can also affected by efficiency of germination.

DETD Increased yield of a transgenic plant of the present invention can be measured in a number of ways, including test weight, seed number per plant, seed weight, seed number per unit area (i.e. seeds, or weight of seeds, per acre), bushels per acre, tonnes per. drought, salt, and attack by pests or pathogens. Recombinant DNA used in this invention can also be used to provide plants having improved growth and development, and ultimately increased yield, as the result of modified expression of plant growth regulators or modification of cell cycle or photosynthesis pathways. Also of interest is the generation of transgenic plants that demonstrate enhanced vield with respect to a seed component that may or may not correspond to an increase in overall plant yield. Such properties include enhancements in seed oil, seed molecules such as tocopherol, protein and starch, or oil particular oil. . DETD

. . . more distal dimerization domain (the K-box) and a C-terminal domain that is usually involved in interactions with other proteins. In plants the region between the MADS box and the K-box has been shown to be important for DNA binding in some. . .

DETD Numerous promoters that are active in plant cells have been described in the literature. These include promoters present in plant genomes as well as promoters from other sources, including nopaline synthase (NOS) promoter and octopine synthase (OCS) promoters carried on. . . actin promoter, U.S. Patent Application Publication 2002/0192813A1, which discloses 5', 3' and intron elements useful in the design of effective plant expression vectors, U.S. patent application Ser. No. 09/757,089, which discloses a maize chloroplast aldolase promoter, U.S. patent application Ser. No.. . maize nicotianamine synthase promoter, all of which are incorporated herein by reference. These and numerous other promoters that function in plant cells are known to those skilled in the art and available for use in recombinant polynucleotides of the present invention to provide for expression of desired genes in transgenic plant cells.

DETD In other aspects of the invention, preferential expression in plant green tissues is desired. Promoters of interest for such uses include those from genes such as Arabidopsis thaliana ribulose-1,5-bisphosphate carboxylase (Rubisco) small subunit (Fischhoff et al. (1992) Plant Mol Biol. 20:81-93), aldolase and pyruvate orthophosphate dikinase (PPDK) (Taniguchi et al. (2000) Plant Cell Physiol. 41(11):42-48).

DETD In other aspects of the invention, sufficient expression in plant seed tissues is desired to effect improvements in seed composition. Exemplary promoters for use for seed composition modification include promoters. . . 1 (Belanger et al (1991) Genetics 129:863-872), glutelin 1 (Russell (1997) supra), and peroxiredoxin antioxidant (Perl) (Stacy et al. (1996) Plant Mol Biol. 31(6): 1205-1216).

DETD . . 3, ocs 3', tr7 3', for example disclosed in U.S. Pat. No. 6,090,627, incorporated herein by reference; 3' elements from plant genes such as wheat (Triticum aesevitum) heat shock protein 17 (Hsp17 3'), a wheat ubiquitin gene, a wheat

fructose-1,6-biphosphatase gene,. . . and the pea (Pisum sativum) ribulose biphosphate carboxylase gene (rbs 3), and 3' elements from the genes within the host plant.

DETD Constructs and vectors may also include a transit peptide for targeting of a gene target to a plant organelle, particularly to a chloroplast, leucoplast or other plastid organelle. For descriptions of

the use of chloroplast transit peptides see.

DETD Transgenic plants comprising or derived from plant cells of this invention transformed with recombinant DNA can be further enhanced with stacked traits, e.g. a crop plant having an enhanced trait resulting from expression of DNA disclosed herein in combination with herbicide and/or pest resistance traits. For. gene from Bacillus thuringensis to provide resistance against lepidopteran, coliopteran, homopteran, hemiopteran, and other insects. Herbicides for which transgenic plant tolerance has been demonstrated and the method of the present invention can be applied include, but are not limited to, . . . Pat. No. 4,810,648 for imparting bromoxynil tolerance; a polynucleotide molecule encoding phytoene desaturase (crtI) described in Misawa et al, (1993) Plant J 4:833-840 and Misawa et al, (1994) Plant J 6:481-489 for norflurazon tolerance; a polynucleotide molecule encoding acetohydroxyacid synthase (AHAS, aka ALS) described in Sathasiivan et al. (1990). . .

DETD

Plant Cell Transformation Methods DETD Numerous methods for transforming plant cells with recombinant DNA are known in the art and may be used in the present invention. Two commonly used methods for plant transformation are Agrobacterium-mediated transformation and microprojectile bombardment. Microprojectile bombardment methods are illustrated in U.S. Pat. Nos. 5,015,580 (soybean); 5,550,318 (corn); . . (cotton); 5,824,877 (soybean); 5,591,616 (corn); and 6,384,301 (soybean), all of which are incorporated herein by reference. For Agrobacterium tumefaciens based plant transformation system, additional elements present on transformation constructs will include T-DNA left and right border sequences to facilitate incorporation of the recombinant polynucleotide into the plant genome.

DETD . . . general it is useful to introduce recombinant DNA randomly, i.e. at a non-specific location, in the genome of a target plant line. In special cases it may be useful to target recombinant DNA insertion in order to achieve site-specific integration, for example to replace an existing gene in the genome, to use an existing promoter in the plant genome, or to insert a recombinant polynucleotide at a predetermined site known to be active for gene expression. Several site. . .

DETD . . gametic cells such as microspores, pollen, sperm and egg cells. It is contemplated that any cell from which a fertile plant may be regenerated is useful as a recipient cell. Callus may be initiated from tissue sources including, but not limited. . . capable of proliferating as callus are also recipient cells for genetic transformation. Practical transformation methods and materials for making transgenic plants of this invention, for example various media and recipient target cells, transformation of immature embryo cells and subsequent regeneration of fertile transgenic plants are disclosed in U.S. Pat. Nos. 6,194,636 and 6,232,526, which are incorporated herein by reference.

The seeds of transgenic plants can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plants line for selection of plants having an enhanced trait. In addition to direct transformation of a plant with a recombinant DNA, transgenic plants can be prepared by crossing a first plant having a recombinant DNA with a second

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plant lacking the DNA. For example, recombinant DNA can be
introduced into first plant line that is amenable to
transformation to produce a transgenic plant which can be
crossed with a second plant line to introgress the recombinant
DNA into the second plant line. A transgenic plant
with recombinant DNA providing an enhanced trait, e.g. enhanced yield,
can be crossed with transgenic plant line having other
recombinant DNA that confers another trait, for example herbicide
resistance or pest resistance, to produce progeny plants
having recombinant DNA that confers both traits. Typically, in such
breeding for combining traits the transgenic plant donating
the additional trait is a male line and the transgenic plant
carrying the base traits is the female line. The progeny of this cross
will segregate such that some of the plants will carry the DNA
for both parental traits and some will carry DNA for one parental trait;
such plants can be identified by markers associated with
parental recombinant DNA, e.g. marker identification by analysis for
recombinant DNA or, in. . . agent such as a herbicide for use with a herbicide tolerance marker, or by selection for the enhanced trait.
Progeny plants carrying DNA for both parental traits can be
crossed back into the female parent line multiple times, for example
usually 6 to 8 generations, to produce a progeny plant with
substantially the same genotype as one original transgenic parental line
but for the recombinant DNA of the other transgenic.
In the practice of transformation DNA is typically introduced into only
a small percentage of target plant cells in any one
transformation experiment. Marker genes are used to provide an efficient
system for identification of those cells. . . markers which confer
resistance to a selective agent, such as an antibiotic or herbicide. Any
of the herbicides to which plants of this invention may be
resistant are useful agents for selective markers. Potentially
transformed cells are exposed to the selective. . .
Plant cells that survive exposure to the selective agent, or
plant cells that have been scored positive in a screening assay,
may be cultured in regeneration media and allowed to mature into
plants. Developing plantlets regenerated from transformed
plant cells can be transferred to plant growth mix,
and hardened off, for example, in an environmentally controlled chamber
at about 85% relative humidity, 600 ppm CO.sub.2, and 25-250
microeinsteins m.sup.-2 s.sup.-1 of light, prior to transfer to a
greenhouse or growth chamber for maturation. Plants are
recenerated from about 6 weeks to 10 months after a transformant is
identified, depending on the initial tissue. Plants may be
pollinated using conventional plant breeding methods known to
those of skill in the art and seed produced, for example
self-pollination is commonly used with transgenic corn. The regenerated
transformed plant or its progeny seed or plants can
be tested for expression of the recombinant DNA and selected for the
presence of enhanced agronomic trait.
Transgenic Plants and Seeds
Transgenic plants derived from the plant cells of
this invention are grown to generate transgenic plants having
an enhanced trait as compared to a control plant and produce
transgenic seed and haploid pollen of this invention. Such
plants with enhanced traits are identified by selection of
transformed plants or progeny seed for the enhanced trait. For
efficiency a selection method is designed to evaluate multiple
transgenic plants (events) comprising the recombinant DNA, for
example multiple plants from 2 to 20 or more transgenic
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events. Transgenic plants grown from transgenic seed provided herein demonstrate improved agronomic traits that contribute to increased yield or other trait that provides increased plant

DETD

DETD

DETD

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value, including, for example, improved seed quality. Of particular interest are plants having enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed.
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- DETD Table 1 provides a list of protein encoding DNA ("genes") that are useful as recombinant DNA for production of transgenic plants with enhanced agronomic trait, the elements of Table 1 are described by reference to:
- DETD . . . number in Table 4 of base vectors used for construction of the transformation vectors of the recombinant DNA. Construction of plant transformation constructs is illustrated in Example 1.

 "PROTEIN NAME" which is a common name for protein encoded by the recombinant.

DETD . . like 1 sequence 1119 PHE0002846_2981 1 Zea Mays trehalose-6-phosphate phosphatase 1120 379 PHE0002864 2999 sov CDKA 8 1121 380 PHE0002869_3004 corn CDKD 12 1122 381 PHE0002875_3010 1 Corn homolog to Arabidopsis unknown expressed protein 1123 382 PHE0002889_3024 soy dsPTP 3 1124 383 PHE0002896 3031 . . . corn AfMONFEED000499 putative indole-3-DETD acetic acid-regulated protein 1482 PHE0004008 4594 4 corn AtMONFEED000474 serine protease-

like protein

Selection Methods for Transgenic Plants with Enhanced Agronomic Trait

DETD Within a population of transgenic plants regenerated from plant cells transformed with the recombinant DNA many plants that survive to fertile transgenic plants that produce seeds and progeny plants will not exhibit an enhanced agronomic trait. Selection from the population is necessary to identify one or more transgenic plant cells that can provide plants with the enhanced trait. Transgenic plants having enhanced traits are selected from populations of plants regenerated or derived from plant cells transformed as described herein by evaluating the plants in a variety of assays to detect an enhanced trait, e.g. enhanced water use efficiency, enhanced cold tolerance, increased yield, . . . surrogate trait. Such analyses can be directed to detecting changes in the chemical composition, biomass, physiological properties, morphology of the plant. Changes in chemical compositions such as nutritional composition of grain can be detected by analysis of the seed composition and. . . acids, oil, free fatty acids, starch or tocopherols. Changes in biomass characteristics can be made on greenhouse or field grown plants and can include plant height, stem diameter, root and shoot dry weights; and, for corn plants, ear length and diameter. Changes in physiological properties can be identified by evaluating responses to stress conditions, for example assays. . . stress conditions such as water deficit, nitrogen deficiency, cold growing conditions, pathogen or insect attack or light deficiency, or increased plant density. Changes in morphology can be measured by visual observation of tendency of a transformed plant with an enhanced agronomic trait to also appear to be a normal plant as compared to changes toward bushy, taller, thicker, narrower leaves, striped leaves, knotted trait, chlorosis, albino, anthocyanin production, or altered. . . include days to pollen shed, days to silking, leaf extension rate, chlorophyll content, leaf temperature, stand, seedling vigor, internode length, plant height, leaf

number, leaf area, tillering, brace roots, stay green, stalk lodging, root lodging, plant health, barreness/prolificacy, green snap, and pest resistance. In addition, phenotypic characteristics of harvested grain may be evaluated, including number of. . . rows of kernels on the ear, kernel abortion, kernel weight, kernel size, kernel density and physical grain quality. Although the plant cells and methods of this invention can be applied to any plant cell, plant, seed or pollen, e.g. any fruit, vegetable, grass, tree or ornamental plant, the various aspects of the invention are preferably applied to corn, sovbean, cotton, canola, alfalfa, wheat and rice plants. In many cases the invention is applied to corn plants that are inherently resistant to disease from the Mal de Rio Cuarto virus or the Puccina sorghi fungus or both.

DETD Plant Expression Constructs

DETD A. Plant Expression Constructs for Corn Transformation

DETD This example illustrates the construction of plasmids for transferring recombinant DNA into plant cells which can be regenerated into transgenic plants of this invention.

DETD A base plant transformation vector pMON65154, as set forth in SEQ ID NO: 52768 was fabricated for use in preparing recombinant DNA for transformation into corn tissue using GATEWAY.TM. Destination plant expression vector systems (available from Invitrogen Life Technologies, Carlsbad, Calif.). With reference to the elements described in Table 3 below, . . . proteinase inhibitor II (pinII) gene. Once recombinant DNA has been inserted into the insertion site, the plasmid is useful for plant transformation, e.g. by microprojectile bombardment.

DETD TABLE 3

FUNCTION ELEMENT REFERENCE

Plant gene of interest Rice actin 1 promoter U.S. Pat. No. 5,641,876

expression cassette Rice actin 1 exon 1, intron 1 U.S.. . . .TM. Cloning Technology

Instruction Manual

ccdA, ccdB genes GATEWAY .TM. Cloning

Technology Instruction Manual

attR2 GATEWAY .TM. Cloning Technology

Instruction Manual

Plant gene of interest Potato pinII 3' region An et al. (1989) Plant Cell 1: 115-122

expression cassette

Plant selectable CaMV 35S promoter U.S. Pat. No. 5,858,742

marker expression nptII selectable marker U.S. Pat. No. 5,858,742 cassette nos 3 region U.S. . . .

A similar base vector plasmid pMON72472 (SEQ ID NO: 52769) was

constructed for use in Agrobacterium-mediated methods of plant transformation similar to pMON65154 except (a) the 5' regulatory DNA in the template recombinant DNA expression cassette was a rice. . .

. . . resistance CR-Ec.aadA-SPC/STR

Repressor of primers from the ColE1 CR-Ec.rop

plasmid Origin of replication

OR-Ec.oriV-RK2 Agro transformation

B-ARGtu.left border Barker, R. F. et al (1983)

Plant Mol Biol 2: 335-350

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Plant selectable marker expression Promoter with intron and
       McDowell et al. (1996)
                                     5'UTR of Arabidopsis act 7
cassette
       Plant Physiol, 111: 699-711.
                                     gene (AtAct7)
                                     5' UTR of Arabidopsis act 7
                                     Intron in 5'UTR of AtAct7
                                    Transit peptide region. . . dicot
      preferred codon
                                    usace
                                    A 3' UTR of the nopaline
                                                                 U.S. Pat.
      No. 5,858,742
                                     synthase gene of
                                     Agrobacterium tumefaciens
                                     Ti plasmid
                                      Promoter for 35S RNA from
  Plant gene of interest expression
       U.S. Pat. No. 5,322,938
                                    CaMV containing a
cassette
                                    duplication of the -90.
DETD
      This example illustrates plant cell transformation methods
       useful in producing transgenic corn plant cells,
       plants, seeds and pollen of this invention and the production
       and identification of transgenic corn plants and seed with an
       enhanced trait, i.e. enhanced water use efficiency, enhanced cold
       tolerance, increased yield, enhanced nitrogen use efficiency, . .
       prepared by cloning DNA identified in Table 1 in the identified base
       vectors for use in corn transformation of corn plant cells to
      produce transgenic corn plants and progeny plants,
       seed and pollen.
DETD
      For Agrobacterium-mediated transformation of corn embryo cells corn
       plants of a readily transformable line (designated LH59) is
      grown in the greenhouse and ears harvested when the embryos are 1.5.
         cells are inoculated with Agrobacterium shortly after excision, and
       incubated at room temperature with Agrobacterium for 5-20 minutes.
       Immature embryo plant cells are then co-cultured with
       Agrobacterium for 1 to 3 days at 23° C. in the dark. Co-cultured
       embryos are. . . develop. Embryogenic callus is transferred to
       culture medium containing 100 mg/L paromomycin and subcultured at about
       two week intervals. Transformed plant cells are recovered 6 to
       8 weeks after initiation of selection.
DETD
       . . . microprojectile bombardment, tissue is cultured in the dark at
       27 degrees C. Additional transformation methods and materials for making
       transgenic plants of this invention, for example, various
       media and recipient target cells, transformation of immature embryos and
       subsequence regeneration of fertile transgenic plants are
      disclosed in U.S. Pat. Nos. 6,194,636 and 6,232,526 and U.S. patent
       application Ser. No. 09/757,089, which are incorporated herein. . .
DETD
      To regenerate transgenic corn plants a callus of transgenic
       plant cells resulting from transformation is placed on media to
       initiate shoot development in plantlets which are transferred to potting
       soil. . . in a growth chamber at 26 degrees C. followed by a mist
       bench before transplanting to 5 inch pots where plants are
       grown to maturity. The regenerated plants are self fertilized
       and seed is harvested for use in one or more methods to select seed,
       seedlings or progeny second generation transgenic plants (R2
       plants) or hybrids, e.g. by selecting transgenic plants
      exhibiting an enhanced trait as compared to a control plant.
DETD
      Transgenic corn plant cells are transformed with recombinant
       DNA from each of the genes identified in Table 1. Progeny transgenic
       plants and seed of the transformed plant cells are
```

screened for enhanced water use efficiency, enhanced cold tolerance,

- increased yield, enhanced nitrogen use efficiency, enhanced seed protein. . .
- DETD This example illustrates plant transformation useful in producing the transgenic soybean plants of this invention and the production and identification of transgenic seed for transgenic soybean having enhanced water use efficiency, enhanced.
- DETD . . Soybean explants and induced Agrobacterium cells from a strain containing plasmid DNA with the gene of interest cassette and a plant selectable marker cassette are mixed no later than 14 hours from the time of initiation of seed germination and wounded. . for an additional two weeks. Roots from any shoots that produce roots off selection are tested for expression of the plant selectable marker before they are transferred to the greenhouse and potted in soil. Additionally, a DNA construct can be transferred into the genome of a soybean cell by particle bombardment and the cell regenerated into a fertile soybean plant as described in U.S. Pat. No. 5,015,580, herein incorporated by reference.
- DETD Transgenic soybean plant cells are transformed with recombinant DNA from each of the genes identified in Table 1. Progeny transgenic plants and seed of the transformed plant cells are screened for enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein.
- DETD of homologs of proteins encoded by the DNA identified in Table 1 which is used to provide transgenic seed and plants having enhanced agronomic traits. From the sequence of the homologs, homologous DNA sequence can be identified for preparing additional transgenic seeds and plants of this invention with enhanced agronomic traits.
- DETD Selection of Transgenic Plants with Enhanced Agronomic Trait(s)
- DETD This example illustrates identification of plant cells of the invention by screening derived plants and seeds for enhanced trait. Transgenic corn seed and plants with recombinant DNA identified in Table 1 were prepared by plant cells transformed with DNA that was stably integrated into the genome of the corn cell. The transgenic seed, plantlets and progeny plants were selected using the methods that measure Transgenic corn plant cells were transformed with recombinant DNA from each of the genes identified in Table 1. Progeny transgenic plants and seed of the transformed plant cells were screened for enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil as compared to control plants.
- DETD The physiological efficacy of transgenic corn plants (tested as hybrids) can be tested for nitrogen use efficiency (NUE) traits in a high-throughput nitrogen (N) selection method. The. . . are due to the transgene. Raw data were analyzed by SAS software. Results shown herein are the comparison of transgenic plants relative to the wildtype controls.
- DETD $\,$ Plants are allowed to grow for 28 days under the low N run or for 23 days under the high N. . .
- DETD (c) Harvest Measurements and Data Collection--After 28 days of plant growth for low N runs and 23 days of plant growth for high N runs, the following measurements are taken (phenocodes in parentheses): total shoot fresh mass (g) (SFM) measured. . . (LDM) measured by Satorius electronic balance. Raw data were analyzed by SAS software. Results shown are the comparison of transgenic plants relative to the wildtype controls.
- DETD . . . readings of corn leaves are affected by the part of the leaf and the position of the leaf on the plant that is sampled, SPAD meter readings were done on leaf six of the plants. Three measurements per leaf were taken, of which the first reading was taken

```
from a point one-half the distance between. . .
DETD
      Level I. Transgenic plants provided by the present invention
      are planted in field without any nitrogen source being applied.
      Transgenic plants and control plants are grouped by
      genotype and construct with controls arranged randomly within genotype
      blocks. Each type of transgenic plants are tested by 3
      replications and across 5 locations. Nitrogen levels in the fields are
      analyzed in early April pre-planting.
DETD
     Level II. Transgenic plants provided by the present invention
      are planted in field with three levels of nitrogen (N) fertilizer being
      applied, i.e. low. . . to the 0 N treatment the soil should still be
      disturbed in the same fashion as the treated area. Transgenic
      plants and control plants are grouped by genotype and
      construct with controls arranged randomly within genotype blocks. Each
      type of transgenic plants is tested by 3 replications and
      across 4 locations. Nitrogen levels in the fields are analyzed in early
      April pre-planting. .
DETD
      Many transgenic plants of this invention exhibit improved
      yield as compared to a control plant. Improved yield can
      result from enhanced seed sink potential, i.e. the number and size of
      endosperm cells or kernels and/or. . .
DETD
        . . has been increasing at a rate of 2.1 bushels/acre/year, but the
      planting density has increased at a rate of 250 plants
      /acre/year. A characteristic of modern hybrid corn is the ability of
      these varieties to be planted at high density. Many studies. .
DETD
      Effective yield selection of enhanced yielding transgenic corn events
      uses hybrid progeny of the transgenic event over multiple locations with
      plants grown under optimal production management practices, and
      maximum pest control. A useful target for improved yield is a 5% to 10%
      increase in yield as compared to yield produced by plants
      grown from seed for a control plant. Selection methods may be
      applied in multiple and diverse geographic locations, for example up to
      16 or more locations, over. . . seasons, for example at least two
      planting seasons to statistically distinguish yield improvement from
      natural environmental effects. It is to plant multiple
      transgenic plants, positive and negative control
      plants, and pollinator plants in standard plots, for
      example 2 row plots, 20 feet long by 5 feet wide with 30 inches distance
      between. . . every two plots to allow open pollination when using
      male sterile transgenic events. A useful planting density is about
      30,000 plants/acre. High planting density is greater than
      30,000 plants/acre, preferably about 40,000 plants
      /acre, more preferably about 42,000 plants/acre, most
      preferably about 45,000 plants/acre. Surrogate indicators for
      yield improvement include source capacity (biomass), source output
      (sucrose and photosynthesis), sink components (kernel size, ear size,.
         . (light response, height, density tolerance), maturity, early
      flowering trait and physiological responses to high density planting,
      for example at 45,000 plants per acre, for example as
      illustrated in Table 10 and 11.
DETD
      . . . 8
Timing
             Evaluation
                                  Description
                                                                comments
V2-3
             Early stand
                                  Can be taken any time after
                                  germination and prior to
                                  removal of any plants.
Pollen shed GDU to 50% shed
                                  GDU to 50% plants shedding
                                  50% tassel.
            GDU to 50% silk
                                  GDU to 50% plants showing
Silking
                                  silks.
Maturity
            Plant height
                                  Height from soil surface to
                                                               10
```

plants per plot - Yield

assistance

Maturity Ear height

plants per plot - Yield

assistance

primary ear attachment node. team

Maturity Leaves above ear visual scores: erect, size, rolling

Maturity Tassel size. . .

DETD . . . is measured with actinic light 1500 (with 10% blue light) micromol m.sup.-2 s.sup.-1, 28° C., CO.sub.2 levels 450 ppm. Ten plants are measured in each event. There were 2 readings for each plant.

flag leaf attachment (inches). team

Height from soil surface to 10

- DETD A hand-held chlorophyll meter SPAD-502 (Minolta--Japan) is used to measure the total chlorophyll level on live transgenic plants and the wild type counterparts a. Three trifoliates from each plant are analyzed, and each trifoliate were analyzed three times. Then 9 data points are averaged to obtain the chlorophyll level. The number of analyzed plants of each genotype ranges from 5 to 8.
- DETD . . . as replications. In this analysis, intra and inter-location variances are combined to estimate the standard error of yield from transgenic plants and control plants. Relative mean comparisons are used to indicate statistically significant yield improvements.
- Described in this example is a high-throughput method for greenhouse DETD selection of transgenic corn plants to wild type corn plants (tested as inbreds or hybrids) for water use efficiency. This selection process imposes 3 drought/re-water cycles on plants over a total period of 15 days after an initial stress free growth period of 11 days. Each cycle consists. . . guenching on the 5th day of the cycle. The primary phenotypes analyzed by the selection method are the changes in plant growth rate as determined by height and biomass during a vegetative drought treatment. The hydration status of the shoot tissues following the drought is also measured. The plant height are measured at three tine points. The first is taken just prior to the onset drought when the plant is 11 days old, which is the shoot initial height (SIH). The plant height is also measured halfway throughout the drought/re-water regimen, on day 18 after planting, to give rise to the shoot. . . height (SMH). Upon the completion of the final drought cycle on day 26 after planting, the shoot portion of the plant is harvested and measured for a final height, which is the shoot wilt height (SWH) and also measured for shoot. . . for four days, the shoots are weighted for shoot dry biomass (SDM). The shoot average height (SAH) is the mean plant height across the 3 height measurements. The procedure described above may be adjusted for +/-.about.one day for each step given. . .
- DEID To correct for slight differences between plants, a size corrected growth value is derived from SIH and SWH. This is the Relative Growth Rate (RGR). Relative Growth. . . each shoot using the formula [RGR %=(SWH-SIH)/((SWH+SIH)/2)*100]. Relative water content (RWC) is a measurement of how much (%) of the plant was water at harvest. Water Content (RWC) is calculated for each shoot using the formula [RWC %=(SWM-SDM)/(SIM-SDM)*100]. Fully watered corn plants of this age run around 99% RWC.
- DETD On the 10.sup.th day after planting the transgenic positive and wild-type negative (WT) plants are positioned in flats in an alternating pattern. Chlorophyll fluorescence of plants is measured on the 10.sup.th day during the dark period of growth by using a PAM-2000 portable fluorometer as per. . . The flats are

- sub-irrigated every day after transfer to the cold temperature. On the 4.sup.th day chlorophyll fluorescence is measured. Plants are transferred to normal growth conditions after six days of cold shock treatment and allowed to recover for the next.
- DETD . . . leaf necrosis and fluorescence during pre-shock and cold shock can be used for estimation of cold shock damage on corn plants
- DETD . . . conventional-till and simulated no-till environments. Seeds are planted into the ground around two weeks before local farmers are beginning to plant corn so that a significant cold stress is exerted onto the crop, named as cold treatment. Seeds also are planted.
- DETD . . . 0 in all plots is also recorded. Seedling vigor is also rated at V3-V4 stage before the average of corn plant height reaches 10 inches, with 1=excellent early growth, 5=Average growth and 9=poor growth. Days to 50% emergence, maximum percent emergence.
- DETD E. Screens for Transgenic Plant Seeds with Increased Protein and/or Oil Levels
- DETD This example sets forth a high-throughput selection for identifying plant seeds with improvement in seed composition using the Infratec 1200 series Grain Analyzer, which is a near-infrared transmittance spectrometer used. . .
- DETD . . . amino acid sequence for the proteins and homologs encoded by DNA that is used to prepare the transgenic seed and plants of this invention having enhanced agronomic traits.
- DETD . . . can be used to identify DNA corresponding to the full scope of this invention that is useful in providing transgenic plants, for example corn and soybean plants with enhanced agronomic traits, for example improved nitrogen use efficiency, improved yield, improved water use efficiency and/or improved growth under cold stress, due to the expression in the plants of DNA encoding a protein

with amino acid sequence identical to the consensus amino acid sequence.

DETD . . . 25 Protein of unknown function (DUF1423)

| DUF1530 DUF1685 | PF07060.1 PF07939.1 | 25 25 | ProFAR isomerase associated Protein of unknown function | | | | |
|--------------------|------------------------|----------|--|--|--|--|--|
| (DUF1685) | FFU/535.1 | 23 | FIOCEIN OI UNKNOWN IUNCCION | | | | |
| DUF246 | PF03138.4 | -15 | Plant protein family | | | | |
| DUF250 | PF03151.6 | 125 | Domain of unknown function, | | | | |
| DUF250 DUF296 | PF03479.4 | -11 | Domain of unknown function | | | | |
| (DUF296) | PF 034/9.4 | -11 | Domain of unknown function | | | | |
| DUF393 | PF04134.2 | 25 | Protein of unknown function, | | | | |
| DUF393 | | | | | | | |
| DUF581 | PF04570.4 | -3.1 | Protein of unknown function | | | | |
| (DUF581) DUF6 | PF00892.9 | 30 | Integral membrane protein DUF6 | | | | |
| DUF641 | PF04859.2 | 25 | Plant protein of | | | | |
| unknown functio | | | Tame process of | | | | |
| | | | (DUF641) | | | | |
| DUF760 | PF05542.1 | 25 | Protein of unknown function | | | | |
| (DUF760) DUF788 | PF05620.1 | 25 | Protein of unknown function | | | | |
| (DUF788) | 1103020.1 | 23 | Trocern or anknown ranceron | | | | |
| Dehydrin | | | .4 20 Kelch motif | | | | |
| Ketoacyl-synt_C | PF02801.10 | -54.9 | Beta-ketoacyl synthase, | | | | |
| C-terminal | | | domain | | | | |
| Kunitz legume | PF00197.8 | -32 | Trypsin and protease inhibitor | | | | |
| LEA_5 | PF00477.7 | 25 | Small hydrophilic | | | | |
| plant seed protein | | | | | | | |
| LIM | PF00412.10 | 0 | LIM domain | | | | |
| LRR_2 | PF07723.2 | 8.7 | Leucine Rich Repeat | | | | |

Lactamase_B PF00753.15 22.3 Metallo-beta-lactamase superfamily PF02866.6 -13 Ldh 1 C lactate/malate dehydrogenase, alpha/beta DETD Selection of Transgenic Plants with Enhanced Agronomic Trait(s) DETD This example illustrates the preparation and identification by selection of transgenic seeds and plants derived from transgenic plant cells of this invention where the plants and seed are identified by screening a having an enhanced agronomic trait imparted by expression of a protein selected from the group including the homologous proteins identified in Example 4. Transgenic plant cells of corn, soybean, cotton, canola, wheat and rice are transformed with recombinant DNA for expressing each of the homologs identified in Example 4. Plants are regenerated from the transformed plant cells and used to produce progeny

plants and seed that are screened for enhanced water use

Plants are identified exhibiting enhanced traits imparted by expression of the homologous proteins.

CLM What is claimed is:

What is claimed is:

23. A plant cell with stably integrated, recombinant DNA comprising a promoter that is functional in plant cells and that is operably linked to DNA encoding a seven-in-absentia protein, wherein said plant cell is present in a plant or seed that (a) exhibits an enhanced trait as compared to control plants that do not have said recombinant DNA; and (b) is derived from a progenitor plant or seed that was selected as having said enhanced trait from a population of plants or seeds that have said recombinant DNA, wherein said enhanced trait is selected from

efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil.

CLM What is claimed is:

25. A plant cell of claim 23 wherein said protein has an amino acid sequence with at least 90% identity to an amino. . .

CLM What is claimed is:
26. A plant cell of claim 23 further comprising DNA expressing
a protein that provides tolerance from exposure to an herbicide applied
at levels that are lethal to a wild type of said plant cell.

CLM What is claimed is: 27. A plant cell of claim 26 wherein the agent of said herbicide is a glyphosate, dicamba, or glufosinate compound.

CLM What is claimed is: 28. A transgenic plant comprising a plurality of the plant cell of claim 23.

CLM What is claimed is: 29. A transgenic seed comprising a plurality of the plant cell of claim 23.

CLM What is claimed is: 30. A transgenic seed of claim 29 from a corn, soybean, cotton, canola, alfalfa, wheat or rice plant.

- CLM What is claimed is: 31. A transgenic pollen grain comprising a haploid derivative of a plant cell of claim 23.
- CLM What is claimed is: 32. A method for manufacturing non-natural, transgenic seed that can be used to produce a crop of transgenic plants with an enhanced trait resulting from expression of stably-integrated, recombinant DNA comprising a promoter that is (a) functional in plant cells and (b) is operably linked to DNA from a plant, bacteria or yeast that encodes a seven-in-absentia protein; wherein said enhanced trait is selected from the group of enhanced traits. . . enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil; wherein said method comprises: (a) screening a population of plants for said enhanced trait and said recombinant DNA, wherein individual plants in said population can exhibit said trait at a level less than, essentially the same as or greater than the level that said trait is exhibited in control plants which do not express the recombinant DNA, (b) selecting from said population one or more plants that exhibit the trait at a level greater than the level that said trait is exhibited in control plants, (c) verifying that said recombinant DNA is stably integrated in said selected plants, (d) analyzing tissue of a selected plant to determine the production of a seven-in-absentia protein in plant cells of claim 23, and (e) collecting seed from a selected plant.

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     02:01:35 ON 11 AUG 2008
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             69 S L2 NOT L1
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     FILE 'USPATFULL' ENTERED AT 02:07:14 ON 11 AUG 2008
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     FILE 'USPATFULL' ENTERED AT 02:07:14 ON 11 AUG 2008
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              1 S L3
L11
             15 S L2
L12
              3 S L11 AND (PLANT OR PLANTS)
L13
              2 S L12 NOT L10
L14
              0 S L8
=> logoff
ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD:v
COST IN U.S. DOLLARS
                                                  SINCE FILE
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FULL ESTIMATED COST
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STN INTERNATIONAL LOGOFF AT 02:09:23 ON 11 AUG 2008